

Kinetics of photochemical isomerization of TFA-Gly-^ZΔPhe into TFA-Gly-^EΔPheMaciej Makowski,^a Michał Jewgiński,^b Józef Hurek,^a Anna Poliwoda,^a and Paweł Kafarski^{a,b}^a Faculty of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland^b Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, PolandEmail: Maciej.Makowski@uni.opole.pl; michal.jewgiński@pwr.edu.pl

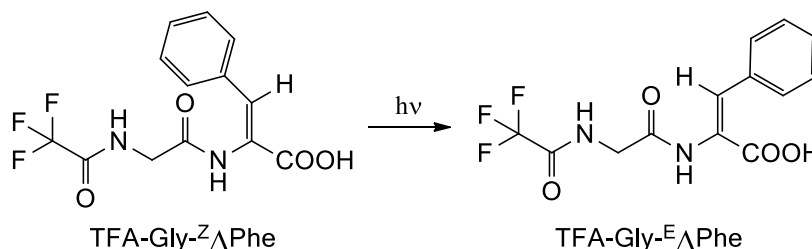
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Abstract

The kinetics of photoisomerization of trifluoroacetyl-(*Z*)-dehydrophenylalanyl-glycine into trifluoroacetyl-(*E*)-dehydrophenylalanyl-glycine was studied in the hope that light-induced reaction could be useful as a means of preparation of the *E*-dehydropeptides. The obtained results indicate that if this reaction carried out under irradiation with light of wavelength 360 nm it is practically irreversible and gave nearly quantitatively pure *E*-isomer. Significantly, expected cyclic side-products were not observed in the reaction mixture, thus proving the preparative potential of the elaborated procedure.

**Keywords:** Dehydropeptides, photoisomerization, *E*-*Z* isomers, reaction kinetics, NMR

Introduction

Dehydroamino acids contribute to catalytic action in tyrosine aminomutase¹ and green fluorescent proteins.² They also occur in a variety of peptide antibiotics of microbial origin (including important lantibiotics such as: nisin, subtilin, epidermin and gallidermin),³ neurotoxins (roquefortin, oxaline and phomopsin),⁴ hepatotoxins (microcystins and nodularins),⁵ and phytotoxic (tentoxin and AM toxins)^{6,7} and antitumor agents (phenylahistin, telomestatin).^{8,9}

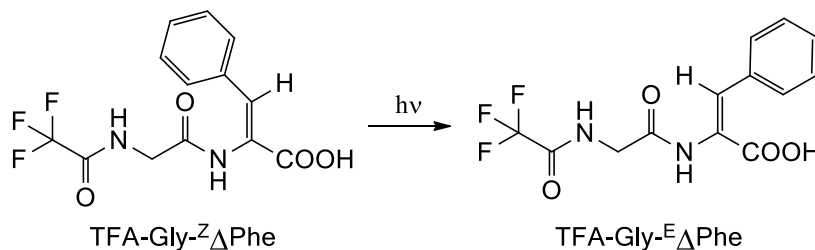
Dehydropeptides might be also considered as a class of promising foldamers¹⁰ because of their ability to take specific three-dimensional structure forms resulting from the presence of a double bond between the C α and C β atoms, which causes the coupling of this double bond with flanking amide fragments and results in rigid structure of the peptide chain.¹¹⁻¹⁴

Numerous studies dealing with dehydropeptide structure and conformation have used ^Z Δ Phe containing peptides owing to their easy chemical synthesis and substantial stability upon storage,¹⁵⁻¹⁸ whereas peptides containing ^E Δ Phe are scarcely described in the literature. This is because *E*-dehydroamino acids and their peptides are unstable and slowly undergo thermal isomerization upon conditions of peptide synthesis.^{19,20}

In this paper we present a study of the kinetics of isomerization of a simple dipeptide derivative containing (*Z*)-dehydrophenylalanine (Gly-^Z Δ Phe) into its *E*-isomer.

Results and Discussion

TFA-Gly-^Z Δ Phe was converted into TFA-Gly-^E Δ Phe (Scheme) by constant irradiation of its benzene/acetone solution with UV light at 254 nm for 18 days at room temperature.



Scheme 1. Photochemical conversion of TFA-Gly-^Z Δ Phe into TFA-Gly-^E Δ Phe.

After fixed periods of time the percent of conversion was determined by means of ¹H NMR spectrometry using the spectra recorded in deuterated DMSO. Three regions of the spectra have been chosen for analysis: (i) intensity of signal derived from ^E Δ Phe amide proton (denoted as A in Figure 1); (ii) intensity of peaks representing amidic protons of ^Z Δ Phe and of two glycines present in both isomers (denoted as B in Figure 1); (iii) intensity of CH₂ region derived from both peptides (denoted as C in Figure 1A). Basing on these measurements relative quantities of both isomers were determined.

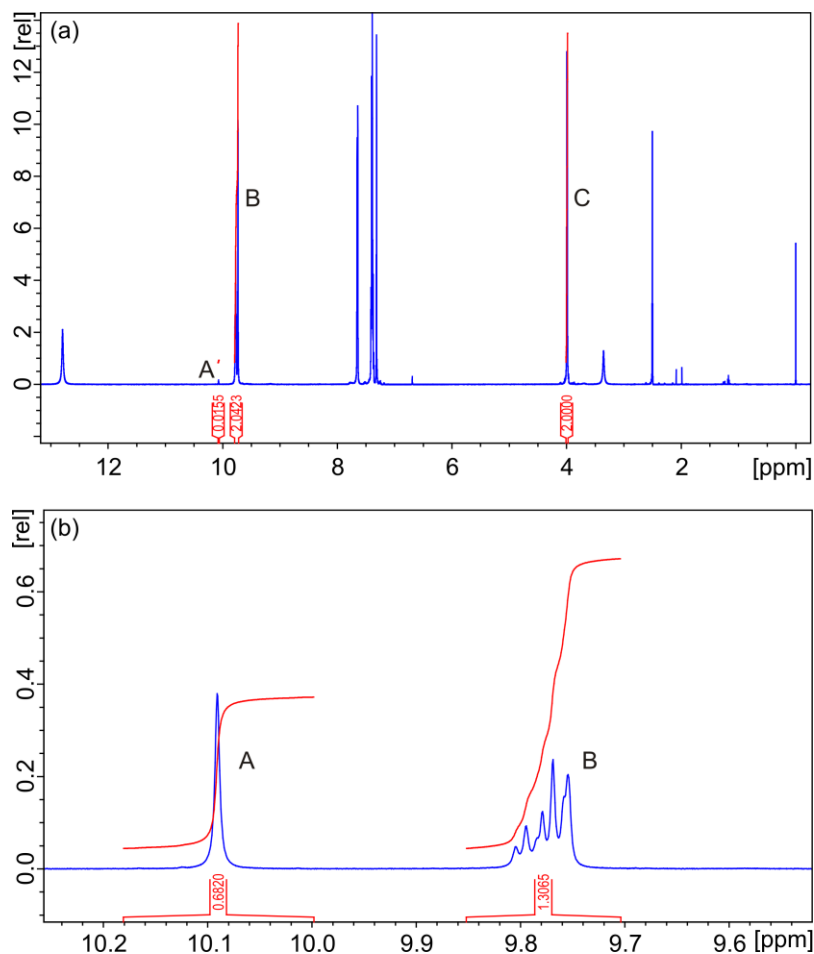


Figure 1. Representative ¹H NMR spectra used to follow the course of isomerization: (a) full spectrum of the reaction mixture at first day of the process; (b) amidic region of spectrum taken after four days of process.

Basing on these determinations curves of progression of the reaction were found (Figure 2) showing quite symmetric increase in molar ratio of isomer *E* in the reaction mixture with simultaneous decrease of the share of isomer *Z*. These progression curves were analyzed by applying standard kinetic equations,²¹ which unequivocally indicated that the studied reaction is of the first order, with k_1 value of $(12.43 \pm 0.27) \times 10^{-3} \text{ h}^{-1}$ and k_{-1} of $(1.71 \pm 0.077) \times 10^{-3} \text{ h}^{-1}$ and $R^2 = 0.996$. Equilibrium of the reaction was reached after fifteen - twenty days of the process. The calculated reaction constant K was 7.26 ± 0.49 indicating seven-fold prevalence of the substrate in final reaction mixture.

To substantiate this finding TFA-Gly-^ZΔPhe was also converted into TFA-Gly-^EΔPhe by constant irradiation of its benzene/acetone solution with light of different wavelength, namely 360 nm, for 80 hours at room temperature. In this case only the decrease in concentration of TFA-Gly-^ZΔPhe was measured by means of HPLC because pure TFA-Gly-^EΔPhe crystallizes from the mixture. Similar as above, analysis of the experimental results (Figure 3) indicated that in this case the reaction is practically irreversible with $k = 0.0458 \pm 0.001943 \text{ h}^{-1}$ and $R^2 = 0.96$. The irreversible character of this reaction clearly and easy isolation of pure TFA-Gly-^EΔPhe in nearly quantitative yield shows that photochemical isomerization of readily available *Z*-dehydropeptides into their *E*-isomers has a preparative value.

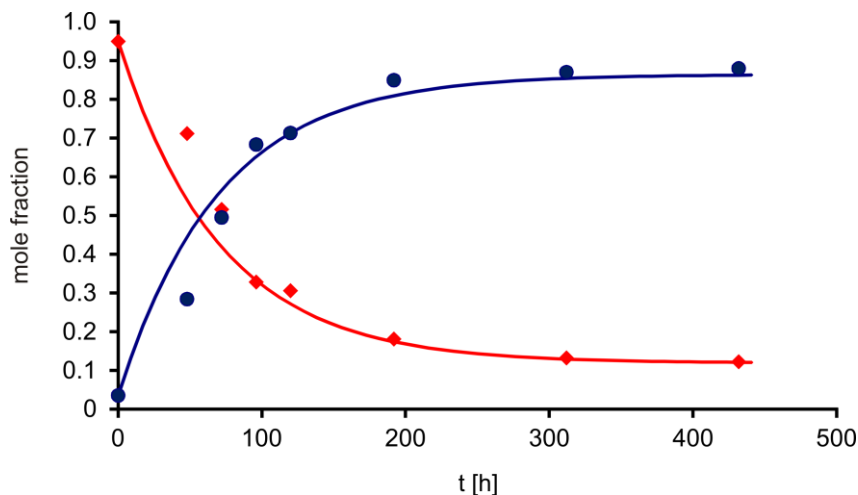


Figure 2. Experimentally (points) and theoretically (lines) determined courses of reaction: increase in concentration of TFA-Gly- $^E\Delta$ Phe (blue line) versus decrease in concentration of TFA-Gly- $^Z\Delta$ Phe (red line).

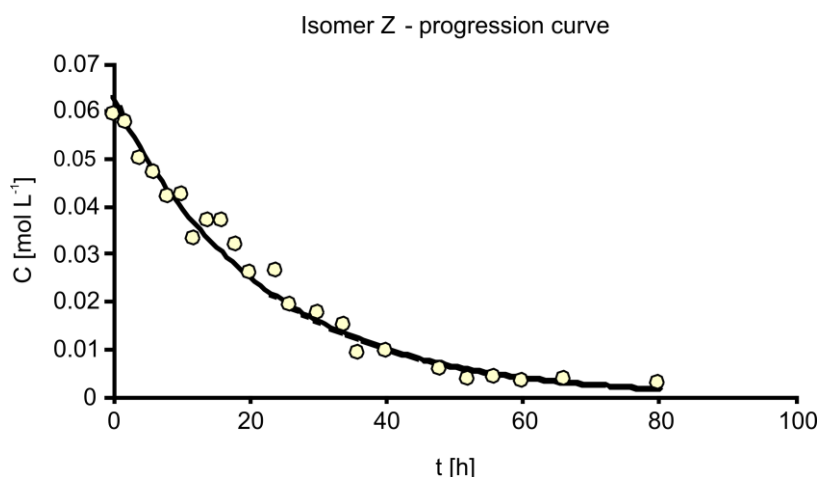


Figure 3. Experimentally (points) and theoretically (line) determined progression curve for conversion of TFA* Δ^Z Phe-Gly.

Preparative conversion of substituted $^Z\Delta$ Phe into $^E\Delta$ Phe,²² as well as such isomerization of this dehydroamino acid in several peptides has been described previously.^{23,24} However, these papers, dedicated to the photochemical reactions of slightly different aromatic dehydroamino acids, state that this process is not so simple and is accompanied by variable photochemical cyclizations.^{25,26} Our study, however, does not indicate the presence of any other products than Gly- $^E\Delta$ Phe in the case of this photochemical reaction, and no cyclization products were found even after long irradiation of reaction mixture, irrespective of which analytical method was used.

Conclusions

Kinetic studies of the photo-conversion of TFA- Δ^Z Phe-Gly into TFA- Δ^E Phe-Gly showed that the equilibrium of process is strongly shifted towards the creation of *E*-isomer. Since the NMR studies did not show the presence of cyclization products, which have been reported in the literature, our studies indicate the preparative

usefulness of the light-induced isomerization of Z-dehydropeptide for obtaining peptides containing the Δ^E Phe residue. This was confirmed by the easy isolation of pure TFA- Δ^E Phe-Gly, which crystallizes from the reaction mixture.

Experimental Section

General. ^1H NMR spectra were recorded on a Bruker Avance II Ultrashield Plus 600 MHz (^1H : 600.58 MHz) using SiMe₄ as internal reference. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hertz respectively. The signal assignments were made using HSCQ spectra.

The samples were analyzed using Waters Model 2695 Alliance Separation Module HPLC System (Waters Corporation, Milford, Massachusetts, USA) equipped with 2487 Dual Absorbance Detector. Data acquisition and integration were performed using Clarity Chromatography software 6.1 (DataApex, Prague, Czech Republic). The studied compounds were separated on Ascentis[®] Express C18 ((100 mm \times 4.6 mm, I.D. 2.7 μm) column protected by a Ascentis[®] Express C18, 2.7 μm guard cartridge (5 mm \times 4.6 mm). The chromatographic separation was performed in isocratic mode, using the mixture of 0.1% CH₃COOH in water (solvent A) and acetonitrile (solvent B) with a ratio 75%A:25%B. The applied flow rate was 0.5 ml min⁻¹. The injection volume was 20 μl . The temperature of the HPLC oven and autosampler was set at 20 $^\circ\text{C}$. The chromatograms were monitored at 280 nm.

Dehydropeptides. Dehydropeptides were available from previous studies.¹²

TFA- Δ^Z Phe-Gly: ^1H NMR(DMSO-*d*₆): δ =3.99 (5.95 2H, d); δ =7.31 (1H, s); δ =7.37÷7.42 (3H, m); δ =7.64÷7.66 (2H, m); δ =9.73 (1H, s); δ =9.77 (6.00 1H, t); δ =12.79 (1H, bs)

TFA- Δ^E Phe-Gly: ^1H NMR(DMSO-*d*₆): δ =3.98 (6.01 2H, d); δ =6.70 (1H, s); δ =7.23÷7.25 (1H, m); δ =7.29÷7.33 (4H, m); δ =9.76 (6.06 1H, t); δ =10.08 (1H, s); δ 13.02 (1H, bs)

Isomerization reaction monitored by ^1H NMR. Trifluoroacetyl-(Z)-glycyl-dehydrophenylalanine (0.050g) was dissolved in a mixture composed of 1.5 ml of benzene and 1.0 ml of acetone. Then 0.112 g of benzophenone (photosensitizer) was added and the reaction mixture irradiated with a 6W lamp at 254 nm. At certain periods of time the sample was evaporated under reduced pressure and its composition studied by ^1H NMR.

Isomerization reaction monitored by HPLC. In this case trifluoroacetyl-(Z)-glycyl-dehydrophenylalanine (3.162g; 10 mmole) was dissolved in mixture of 50 mL of acetone and 95 mL of benzene followed by addition of benzophenone (5.36g; 29.45 mmole) and the mixture irradiated with lamp at 360 nm. At fixed periods of time samples were studied by means of HPLC following the decrease in concentration of the substrate (after filtration of the formed isomer *E*). After reaction crystallized TFA-Gly-^E Δ Phe was separated by filtration (92.6% yield, m.p. 211-213 $^\circ\text{C}$) while the substrate TFA-Gly-^Z Δ Phe was isolated after evaporation of the solvents and crystallization from the mixture of ethyl acetate and hexane (6.2% yield, m.p. 194-196 $^\circ\text{C}$).

Analysis of kinetic data

For the isomerization by irradiation of TFA- Δ^Z Phe-Gly with the wavelength of 254 nm first order reversible kinetic model was assumed as being the most suitable. In order to find kinetic parameters, concentrations of both substrate and product at equilibrium state had to be found. Using the function of linear regression, the best fit between theoretical and experimental curves have been obtained and thus kinetic parameters of the reaction were determined.

Since upon irradiation with the light of wavelength of 360 nm it is irreversible reaction, the first-order

equation was used for its description. Similar procedure yielded its kinetic parameters.

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